Ornamental Horticulture
ISSN 2447-536X | WWW.ORNAMENTALHORTICULTURE.COM.BR
SBFPO Sociedade Brasileira de Floricultura e Plantas Ornamentais

ARTICLE

Enhancement of postharvest performance in *Lilium tigrinum* Ker Gawl flowers with Salicylic acid: a signalling molecule and a growth regulator

Melhoria do desempenho pós-colheita em flores de *Lilium tigrinum* Ker Gawl com ácido salicílico: uma molécula sinalizadora e um regulador de crescimento

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Abstract: The postharvest longevity of *Lilium tigrinum* (Tiger lily) flowers is a critical factor influencing their commercial value, highlighting the need for effective strategies to extend their vase life (VL). This study evaluates the efficacy of salicylic acid (SA) at a concentration of 60 µM as a preservative for prolonging the postharvest life of *L. tigrinum* cut flowers. The results showed that exogenous SA application significantly extended the VL by enhancing relative water uptake, reducing microbial load, and stabilizing various biochemical parameters.SA treatment effectively inhibited lipoxygenase activity, a key enzyme involved in lipid peroxidation, thus mitigating oxidative stress. This protective effect was achieved by boosting the reactive oxygen species (ROS) scavenging capacity, evidenced by increased total phenolic content and elevated activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX). Consequently, membrane lipid peroxidation levels were significantly reduced. Furthermore, SA treatment enhances total soluble protein content, increased proline accumulation, decreased specific protease activity and maintained lower amino acid levels. These enhancements in physiological and biochemical stability can significantly enhance the economic viability of the floral industry by extending the postharvest longevity and maintaining the aesthetic integrity of cut flowers, thereby addressing the increasing global demand for superior-quality floral commodities.
Keywords: antioxidant enzymes, oxidative stress, proline, senescence, vase life.

Resumo: A longevidade pós-colheita das flores de *Lilium tigrinum* (lírio-tigre) influencia diretamente seu valor comercial, destacando a necessidade de estratégias eficazes para prolongar sua vida de vaso (VV). Este estudo avalia a eficácia do ácido salicílico (AS) na concentração de 60 µM como conservante para prolongar a vida pós-colheita de flores cortadas de *L. tigrinum*. Os resultados mostraram que a aplicação exógena de AS estendeu significativamente a VV ao melhorar a absorção relativa de água, reduzir a carga microbiana e estabilizar vários parâmetros bioquímicos. O tratamento com AS inibiu a atividade da lipoxigenase, uma enzima chave envolvida na peroxidação lipídica, mitigando assim o estresse oxidativo. Este efeito protetor foi alcançado ao aumentar a capacidade de eliminação de espécies reativas de oxigênio (ROS), evidenciado pelo aumento do conteúdo fenólico total e das atividades elevadas de enzimas antioxidantes como superóxido dismutase (SOD), catalase (CAT) e ascorbato peroxidase (APX). Consequentemente, os níveis de peroxidação lipídica da membrana foram significativamente reduzidos. Além disso, o tratamento com AS aumentou o conteúdo total de proteínas solúveis, elevou a acumulação de prolina, diminuiu a atividade específica de proteases e manteve níveis mais baixos de aminoácidos. Essas melhorias na estabilidade fisiológica e bioquímica podem aumentar significativamente a viabilidade econômica da floricultura, prolongando a longevidade pós-colheita e mantendo a integridade estética das flores cortadas, atendendo assim à crescente demanda global por produtos florais de qualidade.
Palavras-chave: enzimas antioxidantes, estresse oxidativo, prolina, senescência, vida de vaso.

Introduction

A cascade of morphological, biophysical, and biochemical degradations inherently characterizes flower senescence. In the absence of stringent postharvest handling protocols, cut flowers are prone to rapid desiccation, resulting in a loss of visual appeal (Malakar et al., 2023). Flower senescence is intricately regulated by a network of genetic and physiological mechanisms, encompassing ultrastructural alterations, increased membrane permeability, oxidative stress, and the catabolic breakdown of macromolecules (Lone et al., 2023). This phenomenon poses a significant challenge in the postharvest management of cut flowers. Petal senescence represents the terminal phase of the display life, occurring after physiological maturation and culminating in the apoptotic death of cells, organs, or the entire floral structure. The longevity and quality of cut flowers are critical parameters in the floriculture market (Scariot et al., 2014), ensuring that stakeholders—including wholesalers, retailers and end consumers—remain satisfied and continue their patronage. From a commercial perspective, consumers demand assurance of the ornamental longevity of cut flowers enabling growers to secure premium prices through superior quality and enhanced consumer acceptance (Symoneaux et al., 2022).

Lilium tigrinum, a prominent cut flower, has been documented to undergo senescence through a pathway independent of ethylene (Van Doorn and Woltering, 2004), suggesting the existence of an alternative regulatory system governing the senescence process in *L. tigrinum* flowers, potentially involving oxidative stress mechanisms. The senescence of flower tissues is closely associated with a complex sequence of well-regulated biochemical and physiological processes (Lone et al., 2023). These include elevated respiratory activity, enhanced degradation of macromolecules, increased activity of hydrolytic enzymes, and significant ultrastructural changes in cellular organelles. Notable transformations occur in the tonoplast, with membrane invagination, vacuolar rupture, disintegration of chloroplasts, and alterations in mitochondrial structure (Iakimova et al., 2024).

ROS induce degradation of lipids, proteins, and nucleic acids, leading to cellular death (Ali et al., 2023). Oxidative stress and membrane damage are major contributors to quality loss, particularly in ethylene-insensitive cut flowers (Farooq et al., 2024). Plants possess a sophisticated antioxidative defence system designated to neutralize free radicals, with antioxidant enzymes such as APX, SOD, and CAT playing critical roles in ROS scavenging (Lone et al., 2023). Oxidative stress, coupled with the membrane degradation, is a primary driver of quality deterioration, particularly in ethylene-insensitive flower systems. This underscores the need for effective and economical agents capable of mitigating oxidative stress during the vase life of cut flowers (Arif et al., 2020). Salicylic Acid

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enhances tolerance to abiotic stress through mechanisms such as osmolyte accumulation, secondary metabolite synthesis, increased ROS-scavenging enzyme activity, and improved nutrient uptake (Kaya et al., 2023. Koo et al., 2020).

While salicylic acid (SA) has been shown to effectively delay senescence and extend postharvest longevity in several cut flower species such as *Dianthus caryophyllus* (Dehestani-Ardakani et al., 2022), *Cosmos sulphureus* (Lone et al., 2023), *Consolida ajacis* (ul Haq et al., 2022), by modulating antioxidant system, sugar content, and protein stability, its specific role in *Lilium tigrinum* remains underexplored. The precise mechanisms by which SA influences key physiological and biochemical parameters in *L. tigrinum* cut flowers, such as water relations, oxidative stress responses, and protease activity, have not been comprehensively investigated. Additionally, there is a lack of comparative analysis on the effectiveness of SA across different cut flower species, particularly to variations in flower structure and postharvest physiology. Addressing these gaps that can provide deeper insights into optimizing SA-based postharvest treatments for diverse floricultural crops, thereby contributing to improved vase life and commercial quality.

Material and methods

Healthy and uniform flower buds of *Lilium tigrinum*, grown in the experimental plots at Kashmir University Botanical Garden (KUBG), were harvested at 8:00 a.m. at stage III, defined by partially open buds, one day before anthesis. The cut ends were immediately placed in distilled water and transported to the laboratory. Buds were then divided into five groups for treatment. The control group received no treatment, while the other four groups were treated with varying concentrations of Salicylic acid: 20 µM, 40 µM, 60 µM, and 80 µM. Pedicels were recut to a uniform length of approximately 10 cm, and each bud was placed in a vial covered with aluminium foil, pierced to hold a single bud. The setup was maintained at 27 ± 2 °C, 65% ± 5% relative humidity, with a 12-hour photoperiod. Day 0 marked the start of the experiment, with buds placed in their respective solutions. Assessments of various parameters was conducted on days 2 and 5, with continuous visual monitoring until senescence. The experiment followed a completely randomized design, and data were analyzed using IBM SPSS® Statistics V21.0, with Duncan›s Multiple Range Test (DMRT) used to determine significant differences between treatments ($p < 0.05$).

Measurements

Postharvest flower longevity, solution uptake and Bacterial Density.

Postharvest flower longevity was monitored from the start of treatment until a noticeable decline in aesthetic quality. Solution uptake was determined by measuring the difference between the initial solution volume and the unused volume remaining at the experiment's conclusion. Bacterial density was evaluated by recording the optical density (OD) at 600 nm using a UV-VIS spectrophotometer (Systronics). For this assessment, 1 mL samples were collected from each treatment, including the control. The procedure followed the protocol of Naing et al. (2017), with *E. coli* used as the reference standard (OD 1 = 8×10^8 cfu mL^{-1}).

Quantification of Membrane stability index (MSI)

MSI was indirectly determined through the measurement of solute leakage or conductivity in petal tissues, following the protocol established by Sairam (1994). Conductivity was quantified using an Elico CM180 Conductivity meter.

MSI was calculated using the formula:

MSI = (1-C1/C2) ×100

where C1 and C2 signify the corresponding conductivities at 25 ^{0}C and 100 ^{0}C.

Quantification of proteins and α-amino acids

To quantify soluble proteins, 1 g of petal tissue was ground in a 100 mM phosphate buffer (pH 7.2) The resulting mixture was centrifuged at 12,000 g for 15 minutes at 4 °C. Protein concentration was measured using a suitable aliquot of the supernatant, following the method described by Lowry et al. (1951) with BSA as the standard. α-amino acid content was assessed using Rosen's method (1957) procedure, with glycine used as the standard. Both soluble proteins and α-amino acid content were quantified as mg g^1 fm.

Specific protease activity (SPA)

Petal tissue (1 g) was homogenized in prechilled 0.1 M phosphate buffer (pH 6.5) phosphate buffer (pH = 6.5). After filtration the mixture was centrifuged for 15 minutes at 5000×g at 5 °C. The resulting supernatant served as the enzyme extract for the protease activity assay. After centrifugation, supernatants were collected and free amino acids were quantified as tyrosine equivalents using the method of Tayyab and Qamar (1992). Enzyme activity was expressed as µg tyr mg^{-1} protein.

Quantification of Proline

For estimation of proline one g of plant tissue was homogenised in 4ml of sulphosalicylic acid. Then the homogenate was centrifuged for 10 min at 1,000 rpm, followed by addition of acid Ninhydrin reagent and glacial acetic acid. Proline estimation was performed following the method of Bates et al. (1973), using an appropriate volume of supernatant and L-proline as reference.

Quantification of Antioxidant enzymes Activity

Superoxide dismutase (SOD) activity

SOD activity was measured using the method formulated by Dhindsa et al. (1981), which involves assessing the enzyme's ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). Absorbance was recorded at 560 nm, and SOD activity was quantified as min $^{-1}$ mg^{-1} protein.

Catalase (CAT) activity

It was assessed following the protocol outlined by Aebi (1984), which relies on the enzymatic degradation of H_2O_2. The absorbance was recorded at 240 nm. Enzyme activity was quantified and expressed as micromoles of H_2O_2 reduced min $^{-1}$ mg^{-1} protein

Ascorbate peroxidase (APX) activity

APX activity was evaluated using the method by Chen and Asada (1989), which monitors reduction in absorbance at 290 nm due to ascorbate (0.1 mM) oxidation. Enzyme activity was quantified as μM^{-1} min-^{1} mg $^{-1}$ protein.

Lipoxygenase (LOX) activity

LOX activity was assessed according to the protocol established by Axelrod et al. (1981). Absorbance readings were taken at 234 nm over 5 minutes. The enzymatic activity was quantified and expressed as micromoles of substrate converted min^{-1} mg^{-1} protein.

Quantification of Phenols

To quantify phenols, 1 g of finely chopped tepal tissue was soaked in hot 70% ethanol, macerated, and centrifuged three times. From the resulting supernatant, total phenols were assessed following Swain and Hillis (1959) method with gallic acid as standard.

Results

Visual observations

After anthesis, the detached *L. tigrinum* flowers maintained in distilled water exhibited a lifespan of around five days. Senescence was characterized by petal inrolling and a distinct color transition from vibrant orange to brownish. Importantly, the senescent petals remained attached to the pedicel, showing no abscission (Fig. 1). These laboratory observations of senescence closely resembled those documented in natural field conditions.

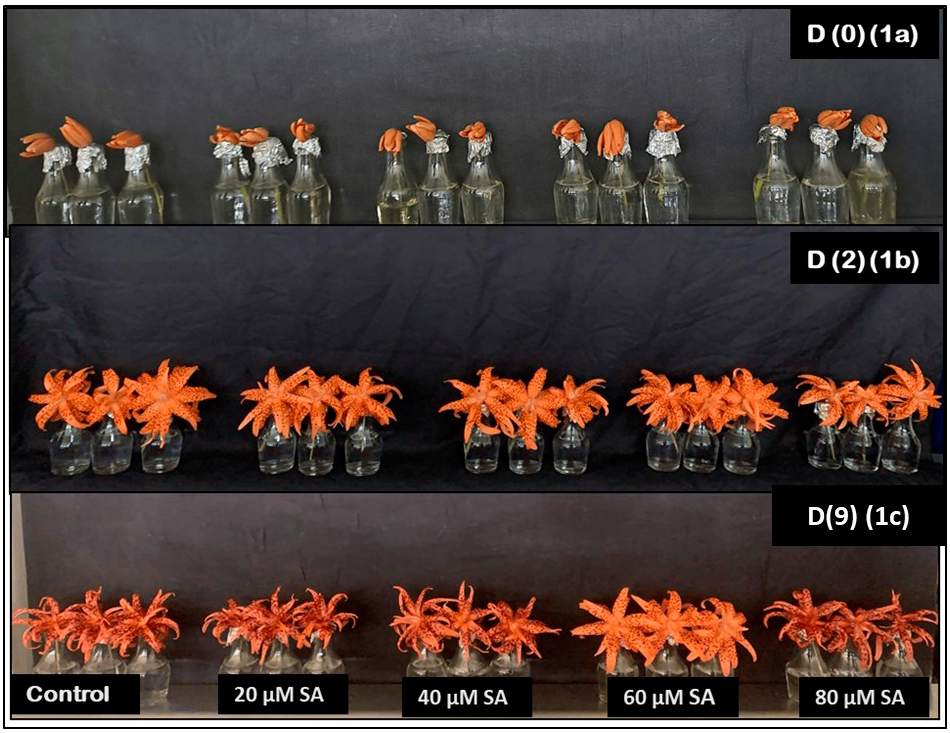

Fig. 1. Effect of different concentrations of Salicylic acid on postharvest performance of isolated flowers of *Lilium tigrinum* on day (0), day 2 (b) and day 9(c).

Post-harvest longevity, Bacterial density and solution uptake

The application of varying concentrations of salicylic acid (SA) significantly extended the postharvest longevity of *L. tigrinum* flowers. The most pronounced effect was seen in flowers treated with 60 µM Salicylic acid, which achieved a lifespan of 9 days. Flowers treated with 80 µM SA exhibited an extended lifespan of 8 days, while those treated with 40 µM and 20 µM SA lasted seven and six days, respectively. In comparison, control flowers placed in distilled water had an average life of only five days (Fig. 2A, 2B, and 2C). Throughout the postharvest evaluation of *L. tigrinum* flowers, the pH of vase solutions was carefully monitored. Solutions supplemented with SA, particularly at 60 µM and 80 µM concentrations, exhibited a significant increase in acidity, with the pH reaching as low as 4.34. In contrast, the control solution maintained a pH of 5.8. This acidification was associated with a decrease in bacterial density in the vase solutions, which correlated with improved solution uptake by the flowers. The highest uptake was observed in flowers treated with 60 µM SA, followed by those treated with 80 µM and 40 µM SA, outperforming the control group.

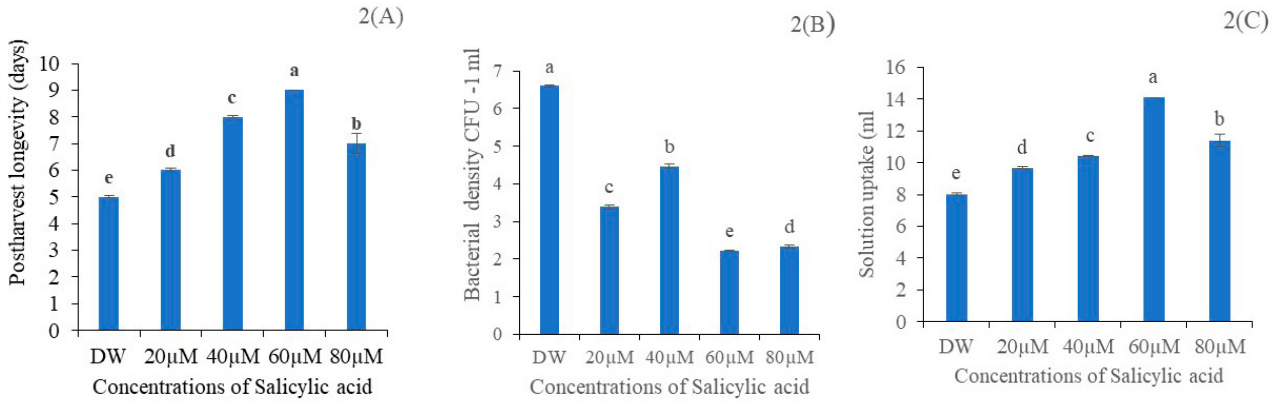

Fig. 2. Graphical representations describing the influence of varying concentrations of Salicylic acid, on the sequential extension of flower longevity (2A), solution uptake (2B) and bacterial density (2C) within the flower tissues of *L. tigrinum* cut flowers. Each data point represents the average of three replicates. Alphabetic symbols positioned above the bars indicate the statistical significance among distinct treatments. Bars distinguished by dissimilar alphabetic symbols denote significant variances according to DMRT ($p < 0.05$).

Variations in antioxidant enzymes and H_2O_2 content

Petals treated with varying concentrations of SA displayed significantly elevated activities of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX). The highest enzymatic activity was observed in flowers treated with 60 µM SA, followed by 80 µM, 40 µM, and 20 µM treatments. However, these enzyme activities markedly declined as the flowers progressed from day 2 to day 5. Additionally, petals treated with 60 µM SA, followed by those treated with 80 µM, 40 µM, and 20 µM, exhibited a notable reduction in hydrogen peroxide (H_2O_2) levels compared to control flowers kept in distilled water, which had the highest H_2O_2 content. Among all treatments, 60 µM Salicylic acid proved to be the most effective in minimizing H_2O_2 accumulation, as depicted in Fig. 3A, 3B, 3C, and 3D.

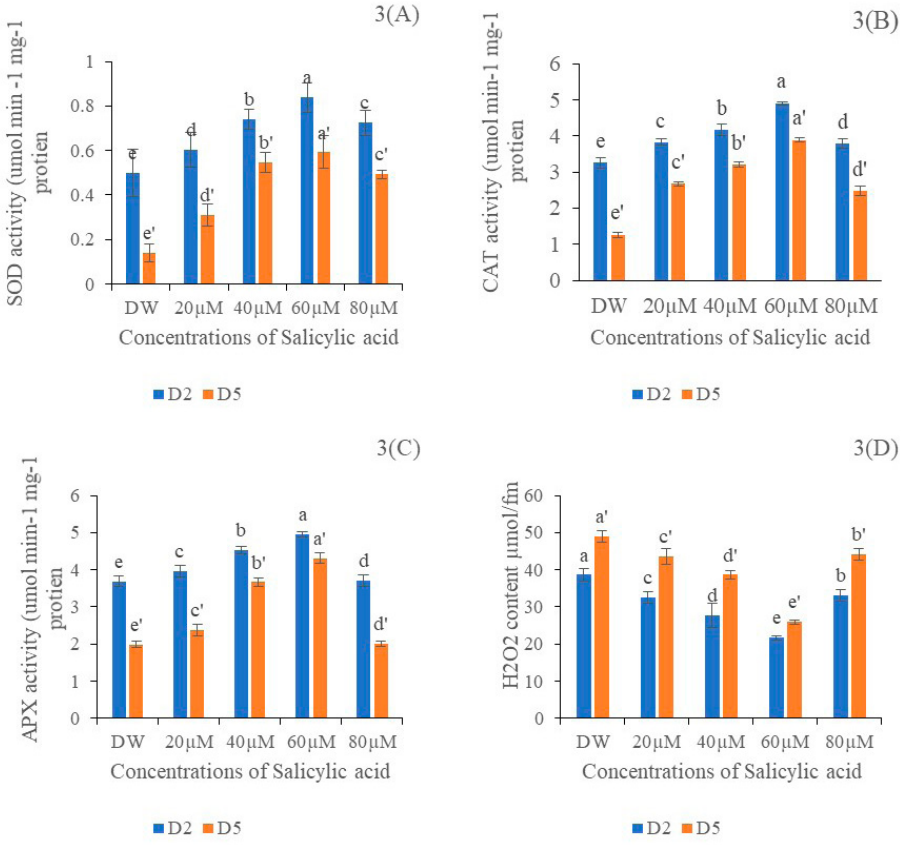

Fig. 3. Graphical representations describing the influence of varying concentrations of Salicylic acid on activities of SOD (A), CAT (B), APX (C) and H_2O_2 content (D) in flowers of *Lilium tigrinum*. Each data point represents the average of three replicates Alphabetic symbols positioned above the bars indicate the statistical significance among distinct treatments. Bars distinguished by dissimilar alphabetic symbols denote significant variances according to DMRT ($p < 0.05$).

MSI and LOX activity

Membrane stability index (MSI) was significantly enhanced in *L. tigrinum* flowers treated with various concentrations of SA, with the highest MSI observed in flowers treated with 60 µM SA. In contrast, lipoxygenase (LOX) enzyme activity in the tepals was substantially reduced, with the lowest activity recorded in flowers treated with 60 µM SA, followed by those treated with 80 µM, 40 µM, and 20 µM SA. Control flowers, by comparison, showed the highest LOX activity and correspondingly lower MSI values. Throughout the treatment, MSI progressively decreased from day 2 to 5, while LOX activity increased, as shown in Fig. 4A and 4B.

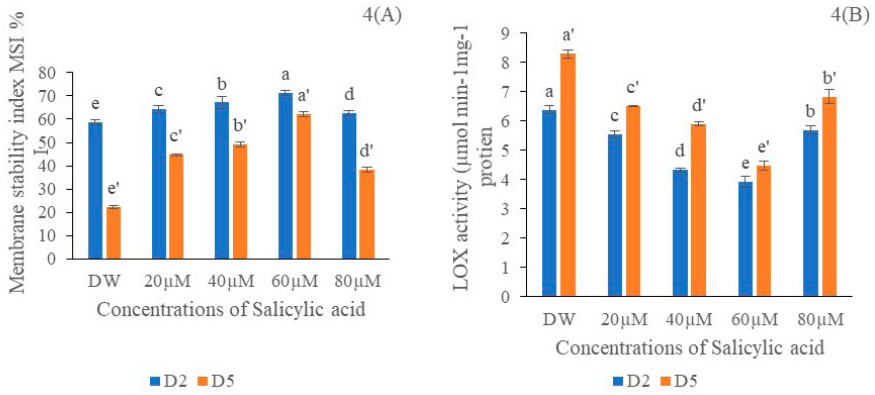

Fig. 4. Graphical representations describing the influence of varying concentrations of Salicylic acid on Lipoxygenase activity (A) and MSI (B) in flowers of *Lilium tigrinum*. Each data point represents the average of three replicates. Alphabetic symbols positioned above the bars indicate the statistical significance among distinct treatments. Bars distinguished by dissimilar alphabetic symbols denote significant variances according to DMRT ($p < 0.05$).

Variations in soluble protein content, α-amino acids, Specific protease Activity, Proline content.

Tepals of *L. tigrinum* flowers treated with 60 µM SA demonstrated a pronounced increase in soluble protein levels, with subsequent increases observed in flowers treated with 80 µM, 40 µM, and 20 µM SA. This elevation in soluble protein content was inversely associated with a significant reduction in specific protease activity (SPA). Control flowers placed in distilled water exhibited the highest SPA, whereas the lowest SPA was recorded in tepals treated with 60 µM SA followed by treatments with 80 µM, 40 µM, and 20 µM SA. Furthermore, tepal tissues exposed to 60 µM SA displayed the lowest levels of α-amino acids, as illustrated in Fig. 5A, 5B, and 5C. Flowers subjected to varying concentrations of salicylic acid (SA) from 20 to 80 mM exhibited a significant increase in proline content. The highest accumulation of proline was observed in petal tissues treated with 60 mM SA depicted in Fig. 5D.

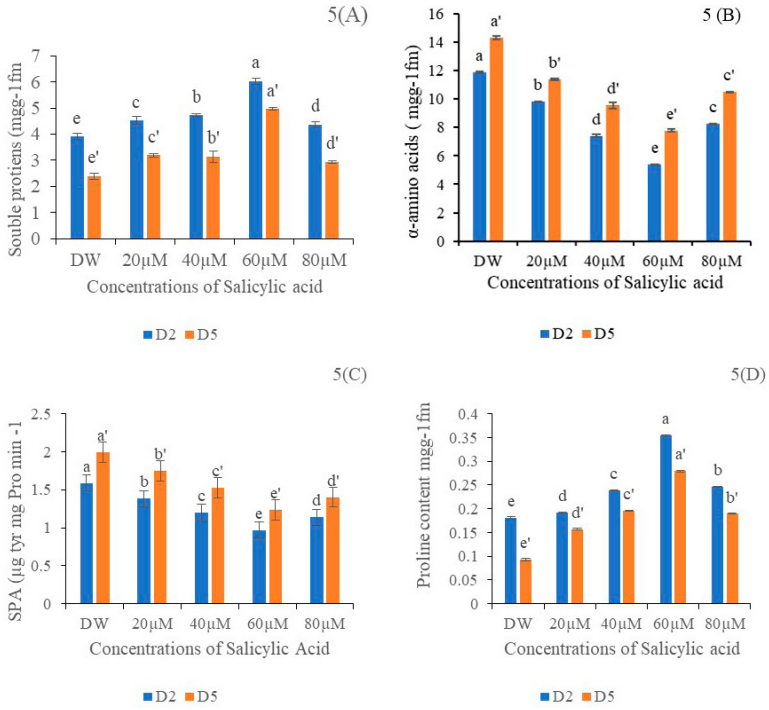

Fig. 5. Graphical representations describing the influence of varying concentrations of Salicylic acid on soluble proteins (A), amino acid content (B), SPA activity (C) and Proline content (D) in flowers of *Lilium tigrinum*. Each data point represents the average of three replicates Alphabetic symbols positioned above the bars indicate the statistical significance among distinct treatments. Bars distinguished by dissimilar alphabetic symbols denote significant variances according to DMRT ($p < 0.05$).

Total phenolic content

Tepals treated with 60 µM SA exhibited the highest concentration of phenolic compounds. The total phenolic content followed a similar trend, with the maximum levels recorded in flowers treated with 60 µM SA on both days 2 and 5, followed by treatments with 80 µM, 40 µM, and 20 µM SA (Fig. 6).

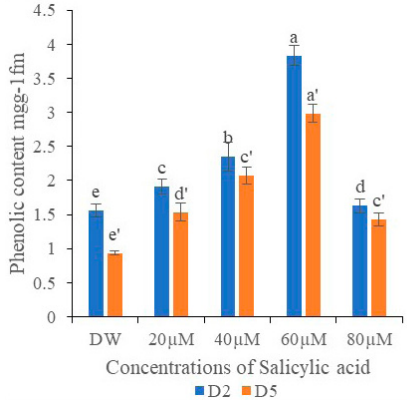

Fig. 6. Graphical representations describing the influence of varying concentrations of Salicylic acid, on total phenols in flowers of *Lilium tigrinum*. Each data point represents the average of three replicates. Alphabetic symbols positioned above the bars indicate the statistical significance among distinct treatments. Bars distinguished by dissimilar alphabetic symbols denote significant variances according to DMRT ($p < 0.05$).

Discussion

Cut flowers are highly valued for their extended longevity, which enhances their commercial and aesthetic appeal (Shinde et al., 2023). A critical determinant of vase life is flower opening, a complex process governed by intricate biological and physiological mechanisms. This process is followed by programmed cell death (PCD), which is essential for floral senescence. Salicylic acid (SA) acts as an endogenous regulator, promoting flowering and modulating key physiological pathways involved in flower development and senescence, thereby enhancing vase life (Hajizadeh et al., 2024). SA treatments that delay flower opening can significantly extend postharvest longevity (ul Haq et al., 2022).

The enhanced solution uptake in cut flowers treated with salicylic acid (SA) is primarily due to its antimicrobial properties, which inhibit pathogen proliferation and prevent microbial contamination (Shi et al., 2024). SA maintains a low pH in vase solutions, reducing ethylene production and minimizing vascular blockages, thereby improving hydraulic conductivity and water transport within flower tissues (Alam et al., 2022). Additionally, SA reduces dehydration by regulating transpiration, increasing tissue turgidity, and promoting petal expansion, thus preserving floral freshness and vitality (Hasanzadeh-Naemi et al., 2021).

SA supports water balance in flowers by promoting the synthesis of osmolytes and metabolites essential for cellular osmoregulation, maintaining cell turgor, and preventing wilting (Shoukat et al., 2023). Additionally, SA enhances the antioxidant defence system, reducing postharvest stress and extending vase life (Ghafari et al., 2020). These physiological and biochemical responses collectively improve water uptake, minimize dehydration, and enhance postharvest quality. Moreover, SA mitigates oxidative damage and preserves membrane integrity by inhibiting lipid peroxidation, as indicated by elevated (MSI) values, highlighting its role in delaying senescence through hormonal regulation (Lone et al.,2023). Salicylic acid (SA) enhances membrane stability and extends postharvest life in cut flowers by reducing oxidative damage and improving antioxidant defences. It lowers malondialdehyde (MDA) levels, a marker of lipid peroxidation, while increasing proline content and boosting antioxidant activity (Lone et al., 2023). This effect has been observed in multiple species, including *Nicotiana plumbaginifolia* (Nisar et al., 2021), *Calendula officinalis* (Lone et al., 2022), and *Cosmos sulphureus* (Lone et al., 2023). SA stabilizes membranes by inhibiting lipoxygenase (LOX) activity and reducing hydrogen peroxide (H_2O_2) accumulation, thereby preventing peroxidative damage to membrane lipids (ul Haq et al., 2022; Lone et al., 2023). Additionally, SA activates the ascorbate-glutathione cycle, further protecting cell membranes and enhancing postharvest longevity (Wang et al., 2023).

Exogenous SA application also stimulates key antioxidant enzymes, including catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX), which collectively detoxify reactive oxygen species (ROS) like H_2O_2, protecting cells from oxidative stress and delaying senescence (ul Haq et al., 2022. Lone et al., 2023). By upregulating stress-responsive genes, SA enhances ROS scavenging and extends the vase life of flowers such as *Consolida ajacis*, *Calendula officinalis*, and *Cosmos sulphureus* (ul Haq et al., 2022. Lone et al., 2022, Lone etal.,2023).

Moreover, SA promotes the synthesis of phenolic compounds, which act as antioxidants by scavenging free radicals and strengthening cell walls. This is achieved through the activation of phenylalanine ammonia-lyase (PAL), a key enzyme in phenolic biosynthesis (Kaya et al., 2023). Increased phenolic content in SA-treated flowers contributes to their resilience against oxidative stress and improves postharvest quality (Lone et al., 2021; Lone et al., 2023).

In addition to floriculture, SA also enhances the quality and decay resistance of fruits such as bayberries, plums, tomatoes, and raspberries by activating similar antioxidant pathways (Wu et al., 2025). These findings underscore SA's potential as a valuable tool for managing oxidative stress and enhancing postharvest longevity in both flowers and fruits.

Increased protease activity and subsequent protein hydrolysis significantly contribute to the degradation of petal tissues (Liu et al., 2022). Conversely, salicylic acid (SA) enhances soluble protein content in flowers, likely by inhibiting protease activity and/or promoting protein synthesis (Nisar et al., 2021). SA may also stimulate the accumulation of osmoprotectants like proline, which stabilizes macromolecules and forms protective hydration shells (Forlani et al., 2019).

Proteins are crucial for maintaining osmotic balance with sugars and enhancing the antioxidant system through the synthesis of stress-specific compounds (Anzano et al., 2022*)*. SA effectively maintains higher protein levels in *Nicotiana*, *Consolida, Calendula, Cosmos* (Nisar et al., 2021 ul Haq et al., 2021, Lone et al., 2022, Lone et al., 2023) which are associated with lower amino acid levels, indicating reduced proteolysis in SA (Lone et al., 2023).

Proline serves as osmoprotectants, maintaining petal turgidity and strengthening antioxidant defences (Parveen et al., 2021). SA also enhances proline accumulation, especially under stress conditions such as drought and salinity. Proline stabilizes proteins, cell membranes, and enzymes while scavenging reactive oxygen species (ROS), thereby reducing oxidative stress (Parveen et al., 2021). This enhancement is achieved by upregulating key enzymes like Δ^1-pyrroline-5-carboxylate synthetase (P5CS) involved in proline biosynthesis and activating stress-responsive pathways. Increased proline content aids osmotic balance, protects cellular integrity, and enhances stress tolerance, ultimately contributing to delayed senescence and prolonged vase life in cut flowers.

Conclusion and future perspectives

This study highlights the efficacy of salicylic acid (SA) in extending the vase life of *Lilium tigrinum* by delaying tepal senescence through mitigating postharvest oxidative stress. SA modulates enzymatic and non-enzymatic defenses by maintaining low lipoxygenase (LOX) activity, increasing phenol, sugar, and protein levels, and enhancing antioxidant enzyme activities. It also inhibits bacterial growth, improving solution uptake. The delayed senescence is further supported by elevated soluble protein and proline content. Future research should focus on optimizing SA application, exploring interactions with other phytohormones, and examining senescence-associated gene expression to establish a molecular basis for improved postharvest management in floriculture.

Author contribution

MA: Writing - Original Draft, Data Curation, Investigation, Visualization and Validation. **HL:** Data Curation, Methodology, Resources and Software**. WWT:** Data Curation, Methodology, Resources and Software. **AAW:** Investigation, Visualization and Formal Analysis. **MAZ**: Investigation, Visualization and Formal Analysis. **IT**: Conceptualization, Resources, Supervision, Writing - Review & Editing

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

Data will be made available on request.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that the use of AI and AI-assisted technologies was not applied in the writing process.

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